Synopsis

Unveiling the Anticariogenic Properties of Medicinal Plants and Development of a Polyherbal Dentifrice

Thesis submitted in partial fulfillment of the degree of Doctor of Philosophy in Botany

By

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Research on oral microbial flora colonization indicates that certain microbiome species decompose dietary polysaccharides, generating organic acids that irreversibly solubilize enamel and dentin minerals, resulting in bacterial biofilm and dental plaque formation, which ultimately causes tooth decay due to an imbalance between acid production and salivary buffering capacities (Featherstone, 2008). Tooth decay progresses to form a hole or cavity in the teeth, causing pain and sensitivity to sweets, heat, and cold. Although the plaque contains diverse microbial species, Streptococcus mutans, a Gram-positive coccus, rapidly metabolizes and produces large amounts of glucosyltransferases (GTFs), greatly aiding adhesion (Banas, 2003). The bacterial surface proteins cause the dental tissue to dissolve, decalcify, and become cavitated.

Fluoride and chlorhexidine, the most effective antiplaque agents, inhibit pathogens' cariogenic activity by disrupting the end cytoplasmic membranes of facultative anaerobes, aerobes, fungi, and both Gram-positive and Gram-negative bacteria (Ceplik et al., 2019). Additional complications include altered taste, tartar development, mucosal irritation, tooth bruising, disruption of the oral microbiota balance, genotoxicity, and oral mucosal cell apoptosis (Ruiz-Linares et al., 2014; Karpinski et al., 2015).

Medicinal plants possessing cariogenic qualities are safe and have negligible adverse effects compared to artificial chemicals. By preventing bacterial growth and the formation of biofilms, compounds derived from plants can aid in the fight against antimicrobial resistance. Beyond preventing dental cavities, they also support oral health by managing conditions like gingivitis and periodontitis and having anti-inflammatory properties (Mohamed et al., 2024) and antioxidant qualities, lowering swelling and

safeguarding oral tissues. These plants not only stop biofilm growth and plaque formation, but they also help to restore enamel's mineral content. These plants exhibit various mechanisms to inhibit *S. mutans*, a primary bacterium responsible for dental caries, including antimicrobial activity, anti-adhesive properties, biofilm disruption, and enzyme inhibition. A limited number of benign antimicrobial substances demonstrate efficacy in curbing the growth of S. mutans by promoting the emergence of symbiotic bacteria, which may contribute to the long-term treatment of dental caries. These substances have been reported to exhibit strong antimicrobial properties, excellent selectivity, and low toxicity (Yang et al., 2021; Atta et al., 2022; Yang et al., 2022).

In resource-constrained communities where synthetic antimicrobials are hard to come by, the incorporation of medicinal plants into oral healthcare presents a viable, affordable, and approachable way to prevent dental caries. Herbal remedies are widely accessible, particularly in rural areas, and are simple to incorporate into oral care routines. Additionally, they support environmentally friendly dental care, which makes them a viable choice. However, more study is required to standardize herbal extracts and formulations, look into the synergistic effects of phytochemicals, and validate safety and efficacy through clinical trials. We can create sustainable, all-natural, and efficient dental health care solutions by utilizing the potential of medicinal plants.

Thus, the present study on **"*Unveiling the Anticariogenic Properties of Medicinal Plants and Development of a Polyherbal Dentifrice*"** is formulated to validate traditional plant-based remedies for oral health, identifying potent inhibitors of *Streptococcus mutans* and to design a novel polyherbal dentifrice with evidenced-based anticariogenic properties.

The objectives framed for the present investigation were

- ➔ To investigate the phytochemical analysis and antioxidant properties of the selected medicinal plants traditionally used for dental caries
- ➔ To evaluate the antimicrobial potential of the selected plant extracts against clinical strains of dental plaque-causing pathogens
- ➔ To identify the potential glucosyltransferase inhibitors from the plant extracts against *S. mutans* using molecular docking
- ➔ To develop and evaluate a Novel PolyHerbal DentiFrice (PHDF) for dental caries
- ➔ To assess the antimicrobial efficacy of the formulated PHDF against oral pathogens by *in vitro* study
- ➔ To validate the anticariogenic effectiveness of PHDF against *S. mutans* by analyzing the compounds and target information to find targets and mechanisms through network pharmacology

Information about medicinal plants used for treating dental cavities was collected from Thottakombai Hill in the Anthiyur region of Erode district, Tamil Nadu. This data represents traditional knowledge of plant-based remedies for tooth decay. The selected plants (AAR-*Achyranthes aspera*, AIL-*Acalypha indica*, AZL-*Azadirachta indica*, APL-*Abrus precatorius*, BCL-*Barleria cuspidata*, EHL-*Euphorbia hirta*, FBP-*Ficus benghalensis*, PBL-*Piper betle*, PGL-*Psidium guajava*, PPL-*Pongamia pinnata*, TPL-*Tridax procumbens*, SVF-*Solanum virginianum*) underwent evaluation to assess their potential anticariogenic effects. Investigation on the qualitative analysis of phytochemical components in the methanol extracts from the chosen plants revealed the presence of various plant-based compounds. The plants *EHL, PBL,* and *PGL* were found to have a greater number of

compounds when compared to other plant extracts. Quantification of the highest amount of alkaloids and terpenoids was found to be present in *Tridax procumbens* leaf (52.37±0.011 mg/g and 19.92±0.017 mg/g). The leaves of *Euphorbia hirta, Tridax procumbens, and Barleria cuspidata* exhibited the highest ascorbic acid concentration of 586.6±0.038 mg/g, 370.9±0.300 mg/g, and 309.0±0.007 mg/g, suggesting its potential as a rich source of vitamin C. The leaves of *Psidium guajava*, *Achyranthes aspera*, *Pongamia pinnata,* and *Solanum virginianum* had the greatest phenolic contents (237.7±0.06, 188.66±0.120, 169.43±0.04, 157.50±0.06, 102.74±0.06 mg GAE/g, respectively), while, *Piper betle, Psidium guajava,* and *Solanum virginianum* fruit had the highest flavonoid contents (228.63±0.08, 201.03±0.121, and 101.912±0.090 mg RE/g, respectively). The extracts of PGL (6.628±6.601 µg/ml), SVF (14.09±5.08 µg/ml), BCL (38.94±0.785 µg/ml), FBP (47.14±20.02), and APL (49.96±0.705) can be classified as extracts with high DPPH° free radical scavenging activity.

The antibacterial screening of the polyherbal crude extracts were performed against *Streptococcus mutans* (SMU)*, Streptococcus salivarius* (SSA)*, Streptococcus oralis* (SOS)*, Streptococcus parasanguinis* (SPSA)*, Klebsiella pneumoniae* (KP)*, Pseudomonas aeruginosa* (PA)*, Acenitobacter baumannii* (AB)*, and Candida albicans* (CA) revealed that all tested bacterial pathogens exhibited a different degree of sensitivity and fluctuated based on the concentration of the extract and the size of the inhibition zone observed for each microorganism tested. Interestingly, the extracts *AAR, EHL, PGL, SVF, BCL, FBP,* and *PBL* showed statistically significant (*p<0.0001*) inhibition against gram-positive isolate *S. mutans* with 24±1.00 mm, 20.3±0.57 mm, 19.6±.20 mm, *and* 18.3±1.52 mm respectively, *S. salivarius* was found to be sensitive towards the extracts SVF, EHL, AAR, PGL, and BCL with 30.3±0.577, 26±4.58, 25.3±0.57, 22.6±1.52, 17.6±1.52 mm zone of inhibition distinctly. For *S. parasanguinis,* the plant extracts EHL, PGL, BCL, and SVF exhibited a

strong zone of inhibition of 22.6±1.52mm, 20.3±0.57mm, 20.0 ±1.00mm, 19.3±0.57mm respectively, and therefore *S. oralis,* the plant extracts PGL, EHL, PBL, AAR, and SVF expressed a 22.6 ±2.52, 21.0±1.00, 20.0±1.00, 19.0±1.00, 18.33±0.57mm zone of inhibition. The gram-negative bacteria *K. pneumoniae* was significantly sensitive to the extracts exhibiting the inhibition zone of 18±1.00, 16.6±1.52, 16±1.00, 15.6±0.57, 15.6±0.57 for FBO, SVF, PGL, EHL ans BCL respectivey while *P. aeruginosa* was resilient to most of the extracts mainly for EHL(27±2.51), PGL(23±2.64), BCL(22.3±3.055), AAR(20.6±1.154), and AIL(20.3±0.577). *A. baumanii* was sensitive to extracts EHL(24±1.00), BCL(23.3±2.08), FBP(23±2.64), PGL(22.6±0.57), AIL(22±1.00), SVF(21.6±1.52), while BCL-(19±1.00), EHL-(19±1. 00), SVF(19±1.00), and APL(18.3±0.57) exhibited greater inhibition against the fungal strain *C.albicans*. The results of IZD revealed that FBP, EHL, BCL, PGL, and AIL were effective against gram-negative isolates, and the fungal isolate was found to be sensitive towards the extracts BCL, EHL, APL, and SVF. The minimal inhibitory concentration (MIC) and minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) were analysed using microbroth dilution method. Of the eight types of oral bacteria examined, Gram-positive *S. mutans, S. salivarius, S. parasanguinis,* and *S. oralis* were the most susceptible to AAR, BCL, EH, FBP, PG, and SVF which showed minimum inhibitory concentrations of 0.625-5 mg/mL, while the MBC ranged 1.25-10 mg/mL for the same. Gram-negative isolates *P. aeruginosa, K. pneumonieae,* and *A. baumanii* were more sensitive towards the plant extracts EHL, PBL, PGL, and SVF when compared to the other plant extracts with the MIC range of 0.3125-2.5 mg/mL. For *C. albicans*, the MIC value of 1.25mg/mL was noted in the plant extracts EHL, FBP, and PBL followed by the lowest MFC value of 2.5 mg/mL. The minimum biofilm eradication of the plant extracts AAR, AP, BCL, EH, FBP, PG, and SVF at the MIC level varied from 47.9 % to 94.9 %. The plant

extracts EH, SVF, and AAR showed significant (*p<0.001*) results exhibiting the biofilm inhibition at the MIC level against gram-positive agents, SMU, SSA, SPSA, and SOS. EHL could inhibit SMU and SSA biofilm formation, displaying 90% & 94.4 % followed by SVF (87% & 93.2%) and AAR (88.4% & 89.2%) respectively. In contrast, extracts BCL, EH, FBP, and PG demonstrated a significant (*p<0.001*; *p<0.005*)) inhibitory effect of 86.2%-93.6% at MIC level (0.002-0.012 mg/ml) against gram-negative agents KP, PA, and AB. The extracts EHL and BCL revealed promising inhibition of 84.5% and 81.2% significantly (*p<0.001*) against the fungal pathogen CA.

The docking studies and molecular dynamics (MD) simulations were carried out for the major bioactive compounds reported from six potential plants (AAR, BCL, EHL, FBP, PGL, SVF) to assess their potential in inhibiting gtfC protein (Glucosyltransferase C), which is associated with the *Streptococcus mutans* (SMU) involved in dental caries. 508 bioactive compounds were initially retrieved, of which 78 compounds were selected using a drug-likeness (DL) score for docking studies. The compounds, Guavin A, stachyurin, Guavin C, Guavin D, Amritoside, 10R, Casuaritcin, Isostrictinin, Guavin B, Procyanidin B3, Tellimargrandin-1, Quercetin-3-O-Gentiobioside, Procyanidin B1, Strictinin, Procyanidin B2, Leucocyanidin, and Goreishic acid from PGL displayed a highest docking score of -14.534, -13.44, -13.388, -11.5203, -11.15, -10.938, -10.862, -9.862, -9.827, -9.606, -9.324, -8.802, -8.736, -8.645, -8.027, and -7.041 kcal/mol respectively towards gtfC. Pentagalloylglucose, 2,4,6-Tri-O-galloyl beta-glucose, Myricitrin, and Kaempferol-3-Glucuronide from EHL produced a strong binding affinity of -11.8495, -11.654, -7.88509, and -7.71528 kcal/mol. The SVF compounds, Solasonine, Esculentin, and Matenoside exhibited a docking score of -9.618, -8.251, and -7.194 kcal/mol respectively. The compounds Proanthocyanidin and 25(S)Ionokesterone derived from AAR showed a docking score of -8.304, and -7.456 kcal/mol followed by

Luteolin-7-O-glucoside with -7.984 kcal/mol from BCL exhibited a high binding affinity towards the gtfC of *S. mutans*. Among these, Guavin A from PGL was further evaluated for stability using an MD simulation study, the RMSD graph revealed stability for 80 ns during the 100 ns simulation period. Guavin A interacted with critical amino acids in the gtfC protein, specifically ARG425, ARG538 (chain B), ARG425, ARG538, and LYS549 (chain F) through ionic bonds, hydrogen bonds, and hydrophobic interactions. The ligand properties confirmed stable and intact interactions, indicating Guavin A's strong potential to inhibit SMU and thereby prevent dental caries. This suggests Guavin A is a promising candidate for further exploration in dental caries prevention through gtfC inhibition.

The six plants namely, AAR, BCL, EHL, FBP, PGL, and SVF were combined in various proportions in the MODDE software, a total of 30 experimental runs using a randomized factorial design was conducted to evaluate combinations of six plant extracts. The PLS model was constructed with an R^2 value of 0.945 which signified a high variation in the response (MIC), and a strong fit between the data and the model. The Minimum Inhibitory Concentration (MIC) values varied from 0.3125 to 5 mg/ml and the PLS model was developed with an R^2 value of 0.947, indicating a strong fit between the experimental data and the model predictions. Selected combinations with a low MIC value of 0.3125 mg/ml against SMU were identified for optimal anti-microbial activity and chosen in the development of formulation. The optimized MIC levels for these plant extracts showed a notable synergistic effect, enhancing their collective antimicrobial efficacy. Pre- and post-compression evaluations showed that the formulated polyherbal dentifrice (Toothpowder tablet), identified as a PHDF, exhibited good mechanical strength with minimal hardness. With the analysis of Atomic Absorption Spectrometric analysis of the formulated PHDF, has no such heavy metals like arsenic, cadmium, lead,

and mercury were found to be present and contained adequate amounts of essential minerals like calcium-7867 mg/kg, and potassium-8209 mg/kg, each contributing to oral hygiene maintenance in various ways. Mortality rate data for the brine shrimp larvae at various observation intervals and concentration levels of PHDF, expressed that PHDF were less toxic in lower and higher concentrations with 17% mortality at 1.5 mg/ml concentration by which the PHDF was considered a safe drug for therapeutic uses. The tablets are designed to release active ingredients upon chewing, facilitating their use for oral hygiene by brushing.

The evaluation of antimicrobial efficacy, the results for the zone of inhibition of formulated polyherbal dentifrice against tested clinical isolates revealed notable differences in effectiveness The PHDF achieved substantial inhibition zones of 21.6±1.15mm for *S. mutans*, 21.0 ±1.00 mm for *S. salivarius*, 21.1±0.57 for *S. parasanguinis*, 21.6±1.52 mm for S*. oralis*, at 150 µg/ml, indicating strong antibacterial activity against the *Streptococcus sp*. The Gram-negative isolates such as *K. pneumoniae, P. aeruginosa,* and *A. baumannii* expressed a ZOI of 22.0±0.00 mm, 23.6±2.3 mm, and 23.6±2.88mm for PHDF respectively. Likewise, the fungal pathogen *C. albicans* exhibited a 20.6±0.57mm ZOI. The ZOI results expressed impartial inhibition against all the tested clinical isolates, demonstrating effective prevention of bacterial growth. The results for the PHDF efficacy against eight pathogens are presented with MIC values ranging from 0.3125 to 1.25 mg/mL and MBC/MFC values ranging from 0.625 to 2.5 mg/mL. A decrease in the hydrophobicity index indicated the downregulation of bacterial adhesion to the enamel. Liability of PHDF at 1/8 MIC, 1/4 MIC, 1/2 MIC, and MIC significantly decreased the hydrophobic indices of the *S. mutans* from 66.7%, 64.3%, 50.5%, 28.5%, and 12.4%, which is slightly effective than the standard NaF (30.8%) at MIC level with a significance range ($P<0.0001$). The activity of PHDF on different MIC values (1/8 MIC, 1/4

MIC, 1/2 MIC, and MIC) on the production of acid by *S. mutans* was examined. The initial pH of all MIC values ranged from 7.3 to 7.5. The pH of the negative control after 24h decreased to 4.0 indicating the acid production of *S. mutans*. PHDF at MIC, showed perpetual pH 7.0 even after 24h of incubation referring to the hindrance of acid production of the cariogenic. A higher dosage (MIC) of PHDF significantly inhibited 94.87% of the biofilm. The results reported that the 1/2 MIC dose of PHDF eradicated 76.87% and half (50%) of the biofilm formed by the cariogenic *S. mutans,* and the initial dose of 1/8 MIC, 1/4 MIC, were able to exterminate only 29.05% and 39.52% of the biofilm, respectively. In addition, the impact of PHDF on the degradation of biofilm was identical to the result obtained with the NaF-positive control with 91% activity, regardless of the concentration with $P<0.0001$ significance. The Polyherbal toothpowder tablets are considered a viable alternative to toothpaste for improving oral hygiene, combining effectiveness with ease of use. The biofilm morphological changes observed by SEM expressed biofilm aggregation in the control sample, after 24h of anaerobic incubation. While, the treatment groups, NaF and PHDF, picturize the disruption of bacterial biofilms. The formulated polyherbal toothpowder tablets were found to be a better alternative to toothpaste to improve oral hygiene. The formulated polyherbal dentifrice (Tooth Tabs) was found to be more effective against major cariogenic microbes than the marketed product, indicating superior performance in preventing dental caries.

From the above results, it was evident that formulated polyherbal toothpowder tablets prevent and cure primary caries by counteracting the growth of caries-causing agents and biofilm. Based on the results from our *in vitro* studies, the bioactive compounds reported in the six plants AAR, BCL, EHL, FBP, PGL, and SVF exhibited a better antimicrobial efficacy towards cariogenic organisms that were included in the

formulation and were selected to examine how the polyherbal and its bioactive components influence dental caries through network pharmacology approach. In addition, we explored the interaction and functional analysis of the target genes involved in dental caries.. The potential 78 compounds were considered the key active compounds responsible for the strong antibacterial activity. To identify the potential interactions of the components, target fishing was done to screen for effective targets (Homosapiens) for all 78 compounds responsible for dental cavities using the SuperPRED 2022 database, the Swiss Target Prediction database, and the disease databases namely Malacards, Comparative Toxicogenomics Database (CTD), and Gene Expression Omnibus Database (GEO). Based on the gift and influence score the targets were filtered and about 24779 targets were retrieved from Mala cards (56), CTD (14373), and GEO (13298) databases related to dental caries. Once the targets were retrieved from these individual databases, we wanted to identify the key targets common to all these databases. Hence, the targets were integrated using the Venny tool, which identified 9 key targets. A PPI (Protein-Protein Interaction) network analysis revealed that 9 major proteins were highly interconnected by 29 edges, indicating significant interactions between them. A total of 78 active compounds were linked to 9 putative targets, forming 516 edges, suggesting these compounds interact with multiple biological targets. The overall network analysis revealed 105 nodes and 596 edges, showing a complex interconnected system of active compounds, targets, and pathways involved in dental caries. These findings suggest that the active biochemical compounds in the formulation may synergistically influence the identified targets involved in dental caries. The pharmacological network analysis reveals that the active compounds in the PHDF target multiple proteins and pathways, creating a "compound-target-pathway" network. This multi-target approach is crucial for its effectiveness. The Polyherbal toothpowder tablets offer a multi-component, multi-target,

and multi-pathway strategy to combat primary dental caries. This broad approach is expected to be effective in preventing dental cavities and related conditions.

This research underscores the importance of integrating traditional knowledge with modern scientific approaches to develop effective, natural, and environmentally friendly solutions for oral health care. The medicinal plants utilized in this research exhibited a high phytochemical profile, and strong effectiveness against cavity-causing microbes isolated from clinical oral samples. The active biochemical compounds in the formulation may synergistically influence the identified targets involved in dental caries. The Polyherbal toothpowder tablets offer a multi-component, multi-target, and multi-pathway strategy to combat primary dental caries. This broad approach is expected to be effective in preventing dental cavities and related conditions.

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